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Biological Sciences

ALLELOPATHY OF WAX MYRTLE *(MYRICA CERIFERA)* ON *SCHINUS TEREBINTHIFOLIUS*

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ABSTRACT: *Nutrient solution leached through soil in which wax myrtle was rooted inhibited growth of schinus. Aqueous leaf extracts of wax myrtle leaves inhibit germination of beans, and possibly of schinus. Allelopathy by wax myrtle reduces the vigor of schinus, and may increase its susceptibility to competitors and pests. Because of the ecological similarity of wax myrtle and schinus, encouragement of myrtle growth may be an effective biological control tool.*

SCHINUS TEREBINTHIFOLIUS Raddi (schinus, Brazilian pepper, Florida holly), of the Anacardiaceae, is an exotic shrub which has been very successful in colonizing many areas in South Florida. It is a sprawling, evergreen, dioecious species, usually 3 to >6 m tall. Females bear bright red berries, 5 mm in dia, which begin to ripen in November; these make the plant highly priced as an oranmental. Seeds are dispersed by several species of birds (Nehrling, 1944) and mammals. The shrub is most abundant on disturbed sites, but is also found in mature plant communities (Duever et al., 1979). Schinus is native to Brazil, Paraguay, and northern Argentina (Krauss, 1963), and is now grown in many other tropical regions as an ornamental. It was introduced to Florida from Brazil around 1892 (Hilsenbeck, 1972) and the seeds were widely distributed for plantings.

The Hole-in-the-Donut section of Everglades National Park is a 6,883 ha area of which 3,644 ha were farmed. Agriculture began in the early 1900's and continued on some parts until July, 1975. Native biotic communities were completely removed from about 2,429 ha through row cropping and rock plowing (Resource Management Staff, 1976). Schinus probably entered the park in the 1940's (Bancroft, 1973), and quickly became established in the Hole-in-the-Donut when agriculture was abandoned. It has formed several dense stands which exclude understory growth and prevent other species from spreading. Once established, the plants survive fire or cold-kill of tops by vigorous basal sprouting.

Attempts to control schinus have met with little success, although some herbicides are effective in killing it. Biological control experimentation with insects native to Brazil and found on schinus there was carried out in Hawaii, where schinus is an important weed of rangelands, with no significant results (Krauss, 1963).

One of us (J. Ewel) is conducting a study of schinus in the Hole-in-the-Donut. One of the sites under investigation is a former pineland, last farmed about 18 yr ago, on which wax myrtle, *Myrica cerifera* L. (Myricaceae), has become dominant. The understory is open, even though there are large schinus trees in the area which serve as a seed source. Measurements indicate a significantly lower growth rate of naturally occurring schinus seedlings in the wax myrtle stand than in the other 4 sites being monitored. These data, plus field observations that showed seedlings to be spindly and unhealthy-looking, led to the hypothesis that wax myrtle might be allelopathic to schinus.

Wax myrtle is a widespread, native, evergreen shrub or small tree found in many environments in Everglades National Park (Craighead, 1971). Wax myrtle often reaches heights of 13 m or more, and grows in association with other woody species (Green, 1939; West and Arnold, 1946). The blue-gray berries, 2 to 3 mm in dia, ripen in September and remain until spring. Birds like them (Green, 1939) and wax from the berries is used by people for medicine, soap, and candles. The leaves and bark are said to have astringent properties (Harper, 1928). The leaves are covered above by minute dark glands, below by orange-colored glands, and have a distinctive balsamic resinous odor when crushed. The trunks may grow to 30 cm in dia and the bark is about 6 mm thick. Wax myrtle bears root nodules containing symbiotic, nitrogen-fixing actinomycetes (Bond, 1976).

Allelopathy is the process by which a plant releases into the environment a chemical compound which inhibits the growth of another plant in the same or a neighboring habitat. It differs from competition in not involving the depletion of a necessary factor in the environment and in depending upon the addition of a deleterious factor (Muller, 1969). Allelopathy may function by one of a number of mechanisms, including auxin oxidation inhibition, the retardation of photosynthesis, and interference with mineral uptake, to name a few. The incidence of chemical inhibition has been demonstrated to be higher in plants from arid regions than in those from humid regions (del Moral and Cates, 1971); however, allelopathy appears to play an important role in altering the structure, function, and diversity of plant communities in many natural and agricultural communities throughout the world.

Experimentation has shown allelopathy to be an important factor affecting plant succession. Certain allelopathic agents inhibit seed germination of some species (Abdul-Wahab and Rice, 1967). Rice (1971) suggested that low-nitrogen-requiring early plant invaders might produce inhibitors of nitrogen-fixing and nitrifying bacteria. He tested 24 species of plants of importance in revegetating old fields against *Azotobacter*, *Rhizobium*, *Nitrobacter*, and *Nitrosomonas*, and found considerable activity was exhibited against most of the test bacteria by 12 plant species.

Several aromatic shrubs were found by Muller, et al. (1964) to produce volatile allelopathic inhibitors. Indications are that these function by rainfall and dew picking up toxic substances from the leaves and transporting them to the ground. Wax myrtle might do the same.

We designed experiments to test the possibility that wax myrtle may produce one or more allelopathic substances that inhibit germination and/or growth of schinus.

Materials and Methods—The effects of wax myrtle exudate on schinus seedlings were investigated using an experimental design modified from Wilson and Rice (1968). Wax myrtle plants, about 0.5 m tall, were obtained from a local nursery. Beans, *Phaseolus vulgaris* L. var. Tendergreen (Fabaceae), were selected as a control because they participate in a symbiotic nitrogen-fixing relationship, as does wax myrtle, and theoretically would have similar nutrient requirements. Schinus seeds were collected from the Hole-in-the-Donut area in December, 1978.

Schinus and beans were planted on 16 January and grown in clean builder's sand with a dilute commercial plant food solution (Ortho 12-6-6). On 11 February the bean plants were transplanted into clean builder's sand in 14 cm dia, glazed clay pots, one plant per pot. On the same day, the wax myrtles were transplanted, one per pot, into the same kinds of pots used for the beans, but the root ball and nursery soil were left intact because we thought they might be a source of allelopathic substances produced by the myrtle. From then until the end of the experiment the beans and wax myrtles were watered and fertilized with a complete nutrient solution (Ross, 1974). On 14 April schinus seedlings were transplanted into clean builder's sand, 3 into each of 14, 10.4 cm dia, glazed clay pots. The experiment began on 16 April and ran for 36 da.

Each myrtle was randomly paired with a bean. Pots containing schinus were randomly assigned to either a wax myrtle or a bean. The experimental setup consisted of 4 tiers of shelving, arranged stair–step fashion to minimize shading, in a greenhouse. The uppermost shelf contained one-liter plastic bottles, one for each myrtle or bean plant, which acted as nutrient-solution reservoirs. The next shelf contained the randomized pairs of pots, wax myrtle alternating with bean. The third shelf contained the pots holding schinus. The lowest shelf contained another set of reservoir bottles. Each container was connected to the plant below it with plastic tubing terminating in a small pipette to insure slow flow. The exteriors of all reservoirs, tubing, and pipettes were painted to inhibit algal growth.

About 900 ml of nutrient solution were placed in the uppermost reservoir, and the flow was adjusted with a screw clamp so that it took about 5 hr for the reservoir to empty. The nutrient solution trickled onto the substrate surface in a pot containing wax myrtle or bean, then flowed out of the bottom of those pots, and trickled down onto the substrate surface in the pots containing schinus. From there it flowed out of the bottom of the schinus pots and was collected in the lower reservoir. Each morning the nutrient solution from each collector was brought back up to 900 ml by adding fresh nutrient solution. The solution was then poured back into the upper reservoir from which it had originated, and the cycle was repeated.

Heights of the schinus and number of leaves per plant were monitored every 2 to 4 da. At the end of the experiment the schinus plants were removed and their stem lengths, root lengths, number of leaves, and oven-dry weights of roots and above-ground parts were recorded. Data were analyzed for each variable using a paired "t" test.

We also tested the effect of aqueous wax myrtle leaf extracts on the germination of schinus seeds. Wax myrtle leaves were collected from the Hole-in-the-Donut on 29 December and stored for 2 mo in a plastic bag. An extract was prepared by boiling 10 g fresh weight of leaves for 5 min in deionized water and grinding in a blender for 10 min at high speed. The solution was allowed to stand for 15 min, then filtered by gravity through Whatman No. 1 paper with a Büchner funnel in a refrigerator. Final volume was brought to 120 ml with deionized water.

Schinus seeds were planted about halfway into about 3 mm of clean builder's sand, with 50 seeds per Petri plate in 20 plates. Ten plates were saturated with leaf extract and 10 with deionized water. The plates were randomly placed on a greenhouse bench. The number of germinated seeds was counted daily and each was removed as its hypocotyl appeared. Two such trials were run.

Experimenters in the past have used fast-growing, easily obtainable plants to quickly test allelopathic effects on germination and growth (e.g., del Moral and Cates, 1971). Beans of the same variety used for the stair-step experiment were chosen for this purpose. Fourteen Petri plates were filled with vermiculite and 5 beans were planted in each plate. Seven randomly chosen plates were moistened with nutrient solution and the other 7 were moistened with wax myrtle extract. The extract was made with leaves obtained from local (Gainesville vicinity) wax myrtle bushes. One liter of cold nutrient solution was added to 50 g fresh weight of leaves and refrigerated for 48 hr. The solution was then strained and stored in a refrigerator overnight, but was warmed to room temperature before application to beans.

Osmolality of the wax myrtle solution was measured with an osmometer (Fiske Model 330 D) and its value was 14 milliosmols/kg, which is too low to have any significant effect on germination by causing osmotic movement of water from the beans into the substrate. The plates were placed in an incubator with a constant humidity of 50%, an 11.5-hr period of darkness with a temperature of 20°C, and a 12.5-hr period of light with a temperature of 30°C. They were removed after 51 hr. Germination rates were noted and hypocotyl lengths of germinated seedlings were measured.

RESULTS AND DISCUSSION—Mean measurements of schinus under wax myrtle were, in all cases, smaller than those under beans (Table 1). Leaf production was significantly ($p < 0.01$) less, with averages of 5.8 schinus leaves under myrtle and 7.3 leaves under beans. Figure 1A shows the pattern of leaf production for the 32 da during which measurements were taken. The most divergence occurred between the day of the first count (day 2) and 18 da afterwards. The greatest growth lag in schinus under wax myrtle occurred at the onset. All schinus seedlings had been transplanted into their pots 2 da before initiation of the experiment; the schinus under wax myrtle may have had more difficulty overcoming transplant shock as a result of the effect of an inhibitory compound. From day 18 to day 30 the leaf productions were about the same, but diverged again during the last 2 days.

TABLE 1. Mean values of characteristics of schinus growing under wax myrtle and under beans. Significant differences (* indicates $p < 0.05$; **indicates $p < 0.01$) were determined using paired, two-tailed "t" test ($n = 7$).

Characteristic of Schinus	Under bean (Control)	Under Wax Myrtle	Difference (Control - Wax Myrtle)
Number of leaves	12.8	11.3	1.5
Leaf production (no./32 days)	7.3	5.8	1.5**
Stem length (mm)	214	189	25
Stem growth (mm/31 days)	122	99	23
Root length (mm)	172	155	17
Stem weight (mg)	47	33	14 **
Root dry weight (mg)	12	7	5
Total dry weight (mg)	59	40	19 *

Height growth followed a similar trend, but for different time periods (Fig. 1B). However, mean values for growth rates of schinus under wax myrtle and under beans (controls) during a 31-da period were not significantly different ($0.5 < p < 0.10$). Stem weights differed significantly ($p < 0.01$), as did total weights ($p < 0.05$), but root lengths, root weights, total numbers of leaves, and stem lengths were not significantly different.

There was an unidentified leaf-spot disease, probably a fungus or a virus, in the schinus plants before they were introduced to the stair-step apparatus. At the end of the experiment the schinus under wax myrtle showed a high incidence of the disease (13 of 21 plants had at least a few spots) and had more insect damage. The schinus under beans showed very little disease;

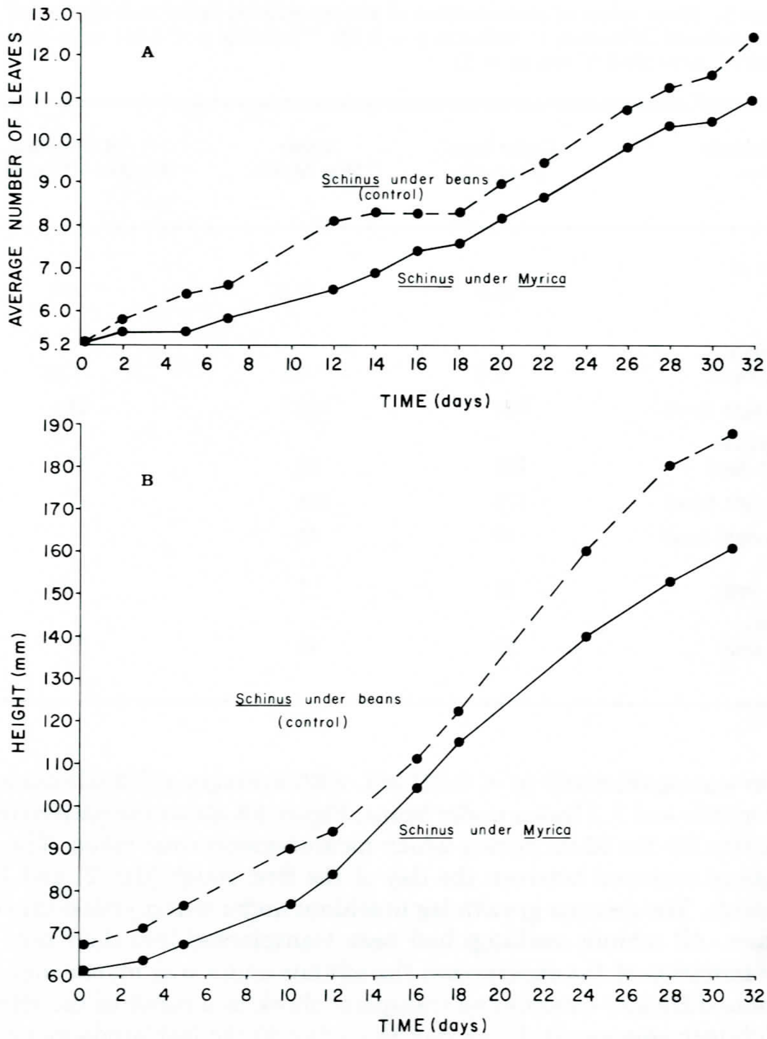

FIG. 1. Growth of schinus seedlings exposed to solution from wax myrtle plants (tests) and from bean plants (controls). Each point is a mean of 21 schinus. A. Leaf production. B. Height growth.

only 2 plants had spots. Inhibition may function by weakening plants, making them more susceptible to disease.

An average of 79 ml more nutrient solution was added to each wax myrtle plant per day than to each bean. This additional amount was necessary because the wax myrtle plants had more roots and more leaf surface than the beans, thus they evapotranspired more. More nutrients may have reached the schinus under wax myrtle, so if any growth stimulation occurred as a result of nutrient availability, it would have been greater under the test plants (myrtle) than under the controls (beans). Nutrient levels were probably not high enough to be toxic.

In the first germination trial, the test plates were treated with leaf extract throughout the experiment. The germination rate of schinus seeds treated with deionized water (controls) was 3.6% and that of seeds treated with wax myrtle extract was 1.2%. In the second trial, wax myrtle extract was administered less often; the sand was kept moist between applications with deionized water in an attempt to increase germination rates in all plates. Germination rates were slightly higher: 4.2% in the control plates and 3.0% in the test plates. Differences were not statistically significant in either trial, however, probably because of the extremely low overall germination. The highest germination rate observed in any Petri plate was 10%, while the average rate in controls for both trials was only 3.9%. The greenhouse environment may not have been humid enough; the Petri plates were not airtight and evaporation from the sand occurred quickly, so the seeds were exposed to daily fluctuations of high moisture followed by dryness. A germination test of 400 schinus seeds from the same 1978-79 seedlot was conducted under environmentally controlled conditions and we observed 21% germination.

Unlike germination of schinus seeds, germination of bean seeds was significantly lower ($p < 0.05$) when they were treated with wax myrtle extract. Germination of controls was 94%; that of beans treated with the extract was only 63%.

The mean hypocotyl length of the bean seeds treated with wax myrtle extract was greater (42.5 mm), but not significantly so, than the mean hypocotyl length of controls treated with nutrient solution (36.3 mm).

Wax myrtle clearly has an allelopathic effect. Although the magnitude of growth inhibition is not as great as that reported for many other species (e.g., Wilson and Rice, 1968), it may be significant in influencing the vigor of species such as schinus in successional South Florida ecosystems. Any reduction in schinus vigor is likely to make it more susceptible to being outcompeted, reduce its reproductive potential, and increase its susceptibility to herbivores and diseases. Schinus and wax myrtle have similar growth forms, and both species are conspicuous invaders of abandoned farmlands in South Florida. Therefore, management practices which favor the development of our native wax myrtle are likely to simultaneously assist in the control of the exotic schinus.

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